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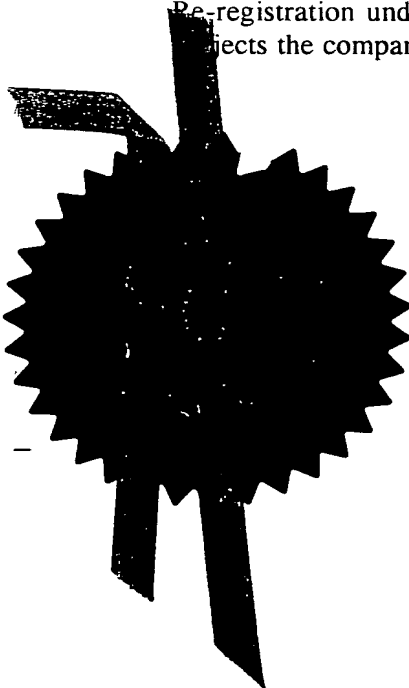
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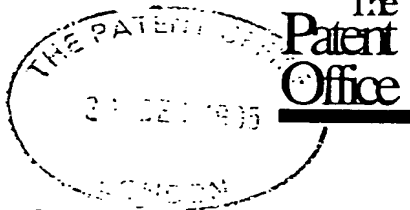
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| 2. Patent application number (The Patent Office will fill in this part) | 21 DEC 1995 / 9526178.0 / | | |
| 3. Full name, address and postcode of the or of each applicant (underline all surnames) | <p>HERMON-TAYLOR, John, DORAN Tim, MILLAR Douglas TIZARD, Mark, LOUGHLIN Mark, SUMAR Nazira and FORD Jon of Department of Surgery, St. George's Hospital Medial School Cranmer Terrace LONDON, SW17 ORE</p> <p>Patents ADP number (if you know it)</p> <p>If the applicant is a corporate body, give the country/state of its incorporation</p> <p>See attached minute sheet for ADP No.</p> | | |
| | UNITED KINGDOM | | |
| 4. Title of the invention | Novel polynucleotides and polypeptides in pathogenic mycobacteria and their use as diagnostics, vaccines and targets for chemotherapy | | |
| 5. Name of your agent (if you have one) | J A KEMP & CO | | |
| "Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode) | 14 SOUTH SQUARE GRAY'S INN LONDON WC1R 5L | | |
| Patents ADP number (if you know it) | 26001 A APPLICATION FILED 20.8.96 | | |
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Date 21 December 1995

12. Name and daytime telephone number of person to contact in the United Kingdom Dr. A.H. Brasnett
0171 405 3292

Novel polynucleotides and polypeptides in pathogenic mycobacteria and their use as diagnostics, vaccines and targets for chemotherapy.

This invention relates to a novel gene which we have identified in pathogenic mycobacteria. These pathogenic mycobacteria also contain a DNA insertion element of the IS900 family such as IS900 itself in *Mycobacterium paratuberculosis* (*Mptb*) IS902 in *M. avium* subsp *silvaticum* (*Mavs*) and a progenitor form of *Mptb* (*Mav* subsp *pp*).

5 Background to the invention

Mycobacterium paratuberculosis (*Mptb*) is a chronic enteric pathogen which can affect many different species of animal including primates. The organism was first identified as the cause of Johne's disease, or 'paratuberculosis' in domestic ruminants. Classically Johne's disease is pluribacillary with millions of bacillary-form acid fast organisms in the affected
10 tissues, together with macrophages, but with little additional granulomatous response. It is now known that there is a paucimicrobial form of 'paratuberculosis' in infected animals in which abundant acid fast *Mptb* cannot be seen, but where there is a chronic granulomatous response. Diagnosis of *Mptb* infection in these animals is often difficult. This spectrum of pluribacillary/ paucimicrobial *Mptb* infection in animals is similar to the spectrum of disease
15 represented by the lepromatous and tuberculoid forms of leprosy in humans. Progress in our understanding of *Mptb* and the diseases it causes has been considerably retarded over the years by the sometimes great difficulty of growing these organisms in the laboratory.

Research using DNA-based detection systems demonstrates that human populations, particularly in developed societies, are being widely exposed to *Mptb* which has undergone
20 amplification in intensely farmed dairy herds. Transmission of this organism to the human population is in the food chain especially milk, and in water draining from heavily grazed pastures. A paucimicrobial chronic enteritis of the Crohn's disease type is initiated in humans exposed unknowingly to this organism, in those individuals with an inherited susceptibility, in people who acquire an intercurrent infection or in people rendered
25 susceptible by their psychological state. *Mptb* infections in animals and humans are the cause of much suffering, as well as large economic losses and health care costs.

The diagnosis of *Mptb* infections in animals is currently still based largely on the uncertainties of conventional faecal culture, as well as serological and cell mediated immune assays whose value is limited by their lack of specificity. Advances have come from DNA-based tests targeting the IS900 family of stably integrated DNA insertion sequences. The
5 overall sensitivity of these procedures is however still inadequate and further improvements in DNA-based testing and in sample preparation are required.

Studies in our own laboratory and subsequently in many others have identified *Mptb* in the chronically inflamed tissues of humans with chronic enteritis of the Crohn's disease type. The diagnosis of this disease in humans is at present based on conventional clinical methods,
10 assisted by costly or invasive procedures including radiology, isotope scanning, endoscopy, biopsy and histopathology. Diagnosis is often delayed sometimes by months or years, with much unnecessary expenditure and suffering. Once the diagnosis is made treatment is at present empirically based on suppressing the immune response. *Mptb* infections in vivo are usually highly resistant to conventional anti-tuberculous drugs. Recent advances in treatment
15 have come from our own laboratory due to the recognition that *Mptb* causes chronic enteritis of the Crohn's disease type in humans and that conventional anti-tuberculous chemotherapy including the aminoglycosides is unlikely to be successful. Chemical modification of natural streptomycetes antibiotics such as the rifamycin series and the macrolides have however produced a current series of drugs such as Rifabutin (Mycobutin), Clarithromycin (Klaricid)
20 and Azithromycin (Zithromax) which are active against Crohn's disease and can induce a profound remission. Resistance of *Mptb* to these agents also can be a problem.

There is a major need for improvements in the diagnosis of *Mptb* infections in humans as well as in animals using both immunological and DNA-based methods. Similar tests are also required for food safety especially milk and dairy products, environmental sampling and
25 laboratory cultures. Advances are needed in the phenotypic characterisation of *Mptb* and in the rapid detection of chemotherapeutic sensitivity or resistance. There is, furthermore, an urgent requirement for effective vaccines for the prevention and treatment of *Mptb* infections in animals and humans as well as for more effective drug treatments.

Description of the Drawings

- Figure 1 shows the identifier DNA sequence (Seq.ID.No1) for the positive strand of SGS1.
- Figure 2 shows the identifier DNA sequence (Seq.ID.No2) for the complementary strand of SGS1.
- Figure 3 shows the identifier amino acid sequences for the polypeptides encoded by the positive strand of SGS1.
- Figure 4 shows the identifier amino acid sequences for the polypeptides encoded by the complementary strand of SGS1.
- Figure 5 shows the specificity of SGS1 for IS900-containing *Mptb*, for IS900-containing *Mav* subsp *pp*. and for IS902-containing *Mavs*.

Disclosure of the invention

Using a DNA-based differential analysis technology we have discovered and characterised a novel polynucleotide in *Mptb* (isolates 0022 from a Guernsey cow and 0021 from a red deer). This polynucleotide is from the gene we have designated SGS1. The gene SGS1 is identified by the corresponding identifier DNA sequences (Seq.ID.No 1 and 2) shown in Figures 1 and 2. SGS1 is present in all isolates of *Mptb*, *Mavs* and in *Mav* subsp *pp*. The complete sequence of the whole gene SGS1 is readily obtainable by a person skilled in the art using all or part of the identifier nucleotide sequences shown in Figures 1 and 2 as probes to screen corresponding genomic libraries, followed by routine DNA sequencing of the corresponding clones. This standard methodology also provides for the identification of the regulatory nucleotide sequences for SGS1 as well as the whole amino acid sequences of the proteins and peptides encoded by the positive and negative strands of SGS1.

The entire functional DNA sequence of the gene SGS1 and its regulatory nucleotide sequences identified as described above, comprise the polynucleotide sequences of the invention.

We have also found that the polynucleotide sequences of the invention comprise open reading frames (ORFs) on both strands of DNA. The proteins and peptides encoded by these ORFs are believed to be associated with specific immunoreactivity and with the pathogenicity of the host microorganisms from which they were obtained, and thus form further aspects of

the invention. The full length amino acid sequences of the proteins and peptides encoded by the positive and negative strands of SGS1 in *Mptb* and *Mavs*, may be determined by the standard methodology described herein.

5 The present invention thus provides a polynucleotide in substantially isolated form capable of hybridizing selectively to the SGS1 gene, said SGS1 gene being obtainable from a pathogenic mycobacteria and characterized by the presence within it of a sequence substantially similar to Seq. ID Nos. 1 or 2.

10 The sequences Seq. ID Nos. 1 and 2 may thus be regarded as "tags" which are characteristic of the SGS1 gene. The presence of such tags, or sequences substantially similar to such tags, in the genome of a pathogenic mycobacteria is indicative of the presence of the SGS1 gene. In general, such tag sequences will be at least 60%, for example at least 70%, 80%, 90% or most preferably 95% or 98% similar (homologous) to Seq. ID Nos. 1 and 2.

15 The present invention also provides a polynucleotide in substantially isolated form which is a fragment of at least 15 or 25 or more than 25 nucleotides of SGS1 or polynucleotides capable of hybridizing selectively thereto. Such polynucleotides are also referred to as polynucleotides of the invention. Polynucleotides of the invention include DNA of Seq. ID Nos 1 and 2 and fragments thereof. The present invention also provides antisense probes capable of hybridising selectively to a polynucleotide of the invention.

20 In a further aspect, the invention provides recombinant vectors carrying a polynucleotide of the invention, including expression vectors, and methods of growing such vectors in a suitable host cell, for example under conditions in which expression of a protein or polypeptide encoded by a polynucleotide of the invention, occurs.

25 In another aspect, the invention provides proteins encoded by the gene SGS1 and homologues thereof, as well as peptide fragments of these proteins, as well as antibodies and synthetic specific ligands identified by standard combinatorial techniques, capable of binding such proteins or peptide fragments thereof. All proteins and peptides within this definition are referred to below as polypeptides of the invention.

In an additional aspect, the invention provides kits comprising polynucleotides, polypeptides, antibodies or synthetic ligands of the invention and methods of using such kits in diagnosing the presence of absence of mycobacteria in a sample.

5 The invention also provides pharmaceutical compositions comprising proteins or peptides of the invention or antisense probes and the use of such compositions in the treatment or prevention of diseases caused by mycobacteria. The invention also provides vaccines for the prevention and treatment of infections due to SGS1-containing pathogenic mycobacteria in animals and humans and as a means of enhancing *in vivo* susceptibility of said mycobacteria to antimicrobial drugs.

10 Detailed description of the invention

A. Polynucleotides

Polynucleotides of the invention as defined herein may comprise DNA or RNA. They may also be polynucleotides which include within them synthetic or modified nucleotides or peptide nucleic acids. A number of different types of modification to oligonucleotides are
15 known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes of the present invention, it is to be understood that the polynucleotides described herein may be modified by any method available in the art. Such modifications may be carried out in order to enhance the recognition, the *in vivo* activity, or the lifespan of
20 polynucleotides of the invention.

The polynucleotides of the invention capable of selectively hybridizing to the DNA of SGS1 will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the corresponding DNA of SGS1 over a region of at least 20, preferably at least 30, for instance at least 40, 60 or 100 or more contiguous nucleotides. Preferred
25 polynucleotides of the invention are those of Seq ID Nos 1 and 2, and fragments thereof.

Any combination of the above mentioned degrees of homology and minimum sizes may be used to define polynucleotides of the invention, with the more stringent combinations (i.e. higher homology over longer lengths) being preferred. Thus for example a polynucleotide

which is at least 80% homologous over 25, preferably 30 nucleotides forms one aspect of the invention, as does a polynucleotide which is at least 90% homologous over 40 nucleotides.

Polynucleotides of the invention may be used to produce a primer, e.g. a PCR primer, a primer for an alternative amplification reaction, a probe e.g. labelled with a revealing label
5 by conventional means using radioactive or non-radioactive labels or a probe linked covalently to a solid phase, or the polynucleotides may be cloned into vectors. Such primers, probes and other fragments will be at least 15, preferably at least 20, for example at least 25, 30 or 40 or more nucleotides in length, and are also encompassed by the term polynucleotides of the invention as used herein. Polynucleotides such as a DNA
10 polynucleotide and probes according to the invention may be produced recombinantly, synthetically, or by any means available to those of skill in the art. They may also be cloned by standard techniques.

In general, primers will be produced by synthetic means, involving a step-wise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing
15 this using automated techniques are readily available in the art. Longer polynucleotides will generally be produced using recombinant means, for example using a PCR (polymerase chain reaction) cloning techniques. This will involve making a pair of primers (e.g. of about 15-30 nucleotides) to a region of SGS1, which it is desired to clone, bringing the primers into contact with genomic DNA from a mycobacterium or a vector carrying the SGS1 sequence,
20 performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the reaction mixture on an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable cloning vector.

25 Such techniques may be used to obtain all or part of the SGS1 sequences described herein, as well as further genomic clones containing full open reading frames. Although in general such techniques are well known in the art, reference may be made in particular to Sambrook J., Fritsch EF., Maniatis T (1989). Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory.

Polynucleotides which are not 100% homologous to the sequences of the present invention but fall within the scope of the invention can be obtained in a number of ways.

Other isolates or strains of pathogenic mycobacteria will be expected to contain allelic variants of the SGS1 sequences described herein, and these may be obtained for example by
5 probing genomic DNA libraries made from such isolates or strains of bacteria using SGS1 sequences as probes under conditions of medium to high stringency (for example 0.03M sodium chloride and 0.03M sodium citrate at from about 50°C to about 60°C).

Alternatively, such polynucleotides may be obtained by site directed mutagenesis of the SGS1 sequences or allelic variants thereof. This may be useful where for example silent codon
10 changes are required to sequences to optimise codon preferences for a particular host cell in which the polynucleotide sequences are being expressed. Other sequence changes may be desired in order to introduce restriction enzyme recognition sites, or to alter the property or function of the polypeptides encoded by the polynucleotides of the invention. Such altered property or function will include the addition of amino acid sequences of consensus signal
15 peptides known in the art to effect transport and secretion of the modified polypeptide of the invention. Another altered property will include mutagenesis of a catalytic residue or generation of fusion proteins with another polypeptide. Such fusion proteins may be with an enzyme, with an antibody or with a cytokine or other ligand for a receptor, to target a polypeptide of the invention to a specific cell type *in vitro*.

20 The invention further provides double stranded polynucleotides comprising a polynucleotide of the invention and its complement.

Polynucleotides or primers of the invention may carry a revealing label. Suitable labels include radioisotopes such as ³²P or ³⁵S, enzyme labels, other protein labels or smaller labels such as biotin. Such labels may be added to polynucleotides or primers of the invention and
25 may be detected using by techniques known per se.

Polynucleotides or primers of the invention or fragments thereof labelled or unlabelled may be used by a person skilled in the art in nucleic acid-based tests for the presence or absence

of *Mptb*, *Mavs*, or other SGS1-containing pathogenic mycobacteria applied to samples of body fluids, tissues, or excreta from animals and humans, as well as to food and environmental samples such as river or ground water and domestic water supplies. Human and animal body fluids include sputum, blood, serum, plasma, saliva, urine, csf, semen, faeces and infected discharges. Tissues include intestine, mouth ulcers, skin, lymph nodes, spleen, lung and liver obtained surgically or by a biopsy technique. Animals particularly include commercial livestock such as cattle, sheep, goats, deer, rabbits but wild animals and animals in zoos may also be tested. Such tests comprise bringing a human or animal body fluid or tissue extract, or an extract of an environmental or food sample, into contact with a probe comprising a polynucleotide or primer of the invention under hybridising conditions and detecting any duplex formed between the probe and nucleic acid in the sample. Such detection may be achieved using techniques such as PCR or by immobilising the probe on a solid support, removing nucleic acid in the sample which is not hybridized to the probe, and then detecting nucleic acid which has hybridized to the probe. Alternatively, the sample nucleic acid may be immobilized on a solid support, and the amount of probe bound to such a support can be detected. Suitable assay methods of this any other formats can be found in for example WO89/03891 and WO90/13667.

Polynucleotides of the invention or fragments thereof labelled or unlabelled may also be used to identify and characterise different strains of *Mptb*, *Mavs*, or other SGS1-containing pathogenic mycobacteria and properties such as drug resistance or susceptibility.

The probes of the invention may conveniently be packaged in the form of a test kit in a suitable container. In such kits the probe may be bound to a solid support where the assay format for which the dit is designed requires such binding. The kit may also contain suitable reagents for treating the sample to be probed, hybridising the probe to nucleic acid in the sample, control reagents, instructions, and the like.

The present invention also provides polynucleotides encoding the polypeptides of the invention described below. Because such polynucleotides will be useful as sequences for recombinant production of polypeptides of the invention, it is not necessary for them to be selectively hybridizable to the sequences Seq. ID Nos 1 and 2, although this will generally

be desirable. Otherwise, such polynucleotides may be labelled, used, and made as described above if desired. Polypeptides of the invention are described below.

B. Polypeptides.

Polypeptides of the invention include polypeptides in substantially isolated form encoded by
5 SGS1. This includes the full length polypeptides encoded by the positive and complementary negative strands of SGS1. Each of the full length polypeptides will contain one of the amino acid sequences set out in Seq.ID.Nos 3 and 4. Polypeptides of the invention further include variants of such sequences, including naturally occurring allelic variants and synthetic variants which are substantially homologous to said polypeptides. In
10 this context, substantial homology is regarded as a sequence which has at least 70%, e.g. 80% or 90% amino acid homology (identity) over 30 amino acids. Polypeptides of the invention may be obtained by the standard techniques mentioned above. Polypeptides of the invention also include fragments of the above mentioned full length polypeptides and variants thereof, including fragments of the sequences set out in Seq.ID Nos 3 and 4. Such
15 fragments for example of 8, 15 or up to 30 or 40 amino acids may also be obtained synthetically using standard techniques known in the art. Preferred fragments include those which include an epitope, especially an epitope which is unique to the mycobacterial cell from which the polypeptide is derived. Suitable fragments will be at least about 5, e.g. 10, 12, 15 or 20 amino acids in size. Epitopes may be determined either by techniques such
20 as peptide scanning techniques as described by Geysen et al, Mol.Immunol., 23; 709-715 (1986), as well as other techniques known in the art.

Polypeptides of the invention may be in a substantially isolated form. It will be understood that the polypeptide may be mixed with carriers or diluents which will not interfere with the intended purpose of the polypeptide and still be regarded as substantially isolated. A
25 polypeptide of the invention may also be in a substantially purified form, in which case it will generally comprise the polypeptide in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the polypeptide in the preparation is a polypeptide of the invention.

Polypeptides of the invention may be modified to confer a desired property or function for example by the addition of Histidine residues to assist their purification or by the addition of a signal sequence to promote their secretion from a cell.

5 A polypeptide of the invention may be labelled with a revealing label. The revealing label may be any suitable label which allows the polypeptide to be detected. Suitable labels include radioisotopes, e.g. ^{125}I , enzymes, antibodies, polynucleotides and ligands such as biotin. Labelled polypeptides of the invention may be used in diagnostic procedures such as immunoassays in order to determine the amount of a polypeptide of the invention in a sample. Polypeptides or labelled polypeptides of the invention may also be used in
10 serological or cell mediated immune assays for the detection of immune reactivity to said polypeptides in animals and humans using standard protocols. A polypeptide or labelled polypeptide of the invention or fragment thereof may also be fixed to a solid phase, for example the surface of an immunoassay well, microparticle, dipstick or biosensor. Such labelled and/or immobilized polypeptides may be packaged into kits in a suitable container
15 along with suitable reagents, controls, instructions and the like.

Such polypeptides and kits may be used in methods of detection of antibodies or cell mediated immunoreactivity, to the mycobacterial proteins and peptides encoded by the SGS1 and their allelic variants and fragments, using immunoassay. Such host antibodies or cell mediated immune reactivity will occur in humans or animals with an immune system which
20 detects and reacts against polypeptides of the invention. The antibodies may be present in a biological sample from such humans or animals, where the biological sample may be a sample as defined above particularly blood or saliva.

Immunoassay methods are well known in the art and will generally comprise:

- 25 (a) providing a polypeptide of the invention comprising an epitope bindable by an antibody against said mycobacterial polypeptide.
- (b) incubating a biological sample with said polypeptide under conditions which allow for the formation of an antibody-antigen complex; and

- (c) determining whether antibody-antigen complex comprising said polypeptide is formed.

Immunoassay methods for cell mediated immune reactivity in animals and humans are also well known in the art (e.g. as described by Weir et al 1994, *J.Immunol Methods* 176; 93-
5 101) and will generally comprise

- (a) providing a polypeptide of the invention comprising an epitope bindable by a lymphocyte or macrophage or other cell receptor
- (b) incubating a cell sample with said polypeptide under conditions which allow for a cellular immune response such as release of cytokines or other mediator
10 to occur; and
- (c) detecting the presence of said cytokine or mediator in the incubate.

Polypeptides of the invention may be made by standard synthetic means well known in the art or recombinantly, as described below.

C. Vectors.

- 15 Polynucleotides of the invention can be incorporated into a recombinant replicable vector. The vector may be used to replicate the nucleic acid in a compatible host cell. Thus in a further embodiment, the invention provides a method of making polynucleotides of the invention by introducing a polynucleotide of the invention into a replicable vector, introducing the vector into a compatible host cell, and growing the host cell under conditions
20 which bring about replication of the vector. The vector may be recovered from the host cell. Suitable host cells are described below in connection with expression vectors.

D. Expression Vectors.

- Preferably, a polynucleotide of the invention in a vector is operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host
25 cell, i.e. the vector is an expression vector. The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence

is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. Such vectors may be transformed into a suitable host cell as described above to provide for expression of a polypeptide of the invention. Thus, in a further aspect the invention provides a process for preparing polypeptides according to the invention which comprises cultivating a host cell transformed or transfected with an expression vector as described above, under conditions to provide for expression by the vector of a coding sequence encoding the polypeptides, and recovering the expressed polypeptides.

A further embodiment of the invention provides vectors for the replication and expression of SGS1, or fragments thereof. The vectors may be for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin resistance gene in the case of a bacterial plasmid or a neomycin resistance gene for a mammalian vector. Vectors may be used *in vitro*, for example for the production of RNA or used to transfect or transform a host cell. The vector may also be adapted to be used *in vivo*, for example in a method of naked DNA vaccination or gene therapy. A further embodiment of the invention provides host cells transformed or transfected with the vectors for the replication and expression of polynucleotides of the invention, including the DNA of SGS1 and the open reading frames thereof. The cells will be chosen to be compatible with the said vector and may for example be bacterial, yeast, insect or mammalian. Polynucleotides according to the invention may also be inserted into the vectors described above in an antisense orientation in order to provide for the production of antisense RNA. Antisense RNA or other antisense polynucleotides or ligands may also be produced by synthetic means. Such antisense polynucleotides may be used in a method of controlling the levels of the proteins encoded by SGS1 in a mycobacterial cell.

E. Antibodies

The invention also provides monoclonal or polyclonal antibodies to polypeptides of the invention or fragments thereof. The invention further provides a process for the production of monoclonal or polyclonal antibodies to polypeptides of the invention. Monoclonal

antibodies may be prepared by conventional hybridoma technology using the polypeptides of the invention or peptide fragments thereof, as immunogens. Polyclonal antibodies may also be prepared by conventional means which comprise inoculating a host animal, for example a rat or a rabbit, with a polypeptide of the invention or peptide fragment thereof and
5 recovering immune serum.

In order that such antibodies may be made, the invention also provides polypeptides of the invention or fragments thereof haptenised to another polypeptide for use as immunogens in animals or humans.

For the purposes of this invention, the term "antibody", unless specified to the contrary,
10 includes fragments of whole antibodies which retain their binding activity for a polypeptide of the invention. Such fragments include Fv, F(ab') and F(ab')₂ fragments, as well as single chain antibodies. Furthermore, the antibodies and fragments thereof may be humanised antibodies, e.g. as described in EP-A-239400.

Antibodies may be used in methods of detecting polypeptides of the invention present in
15 biological samples (where such samples include the human or animal body samples, and environmental samples, mentioned above) by a method which comprises:

- (a) providing an antibody of the invention;
- (b) incubating a biological sample with said antibody under conditions which allow for the formation of an antibody-antigen complex; and
- 20 (c) determining whether antibody-antigen complex comprising said antibody is formed.

Antibodies of the invention may be bound to a solid support for example an immunoassay well, microparticle, dipstick or biosensor and/or packaged into kits in a suitable container along with suitable reagents, controls, instructions and the like.

25 F. Compositions.

The present invention also provides compositions comprising a polynucleotide or polypeptide of the invention together with a carrier or diluent. Such compositions include

pharmaceutical compositions in which case the carrier or diluent will be pharmaceutically acceptable.

Pharmaceutically acceptable carriers or diluents include those used in formulations suitable for inhalation as well as oral, parenteral (e.g. intramuscular or intravenous or transcutaneous) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

For example, formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the polynucleotide or the polypeptide of the invention to blood components or one or more organs.

G. Vaccines.

In another aspect, the invention provides novel vaccines for the prevention and treatment of infections caused by *Mtpb*, *Mavs* and other SGS1-containing pathogenic mycobacteria in animals and humans. Such vaccines include polynucleotides of the invention or fragments thereof in suitable vectors and administered by injection of naked DNA using standard protocols. Polynucleotides of the invention or fragments thereof in suitable vectors for the expression of the polypeptides of the invention may be given by injection, inhalation or by mouth. Suitable vectors include *M.bovis* BCG, *M.smegmatis* or other mycobacteria, *Corynebacteria*, *Salmonella* or other agents according to established protocols. Polypeptides of the invention or fragments thereof in substantially isolated form in doses approximately 5-20mg/Kg body weight may be used as vaccines by injection, inhalation, or transcutaneous application according to standard protocols. Adjuvants, cytokines such as IL-12 and other

immunomodulators may be used for the selective enhancement of the cell mediated or humoral immunological responses. Vaccination with polynucleotides and/or polypeptides of the invention may be undertaken to increase the susceptibility of SGS1-containing pathogenic mycobacteria to antimicrobial agents *in vivo*.

- 5 The following Example illustrates the invention.

EXAMPLE 1

Tests for the presence of SGS1 were performed on 5 μ l bacterial DNA extracts (25 μ g/ml to 500 μ g/ml) using polymerase chain reaction based on the oligonucleotide primers 5'-GATGCCGTGAGGAGGTAAAGCTGC-3' and 5'-GATACGGCTCTTGAATCCTGCACG-3' from within the identifier DNA sequences (Seq.ID.Nos 1 and 2) shown in Figures 1 and 2. PCR was performed for 40 cycles in the presence of 1.5 mM magnesium and an annealing temperature of 58°C. The presence + or absence - of the correct amplification product indicated the presence or absence of the SGS1 gene in the corresponding bacterium. SGS1 is shown to be present in all the laboratory and field strains of *Mptb* and *Mavs* tested, as well as in a clinical isolate of pathogenic *Mav* subsp *pp*. SGS1 is absent from other mycobacteria as well as from other organisms. IS900 PCR was performed as described by Millar *et al* 1995 Analyt Biochem 226; 325-330 and IS902 PCR as described by Moss *et al* 1992 GUT 33; 1209-1213. As before, the presence + or absence - of the correct amplification product indicated the presence or absence of the element associated with pathogenicity in the corresponding mycobacterium.

CLAIMS

1. A polynucleotide in substantially isolated form capable of hybridizing selectively to the SGS1 gene, said SGS1 gene being obtainable from a pathogenic mycobacteria and characterized by a sequence substantially similar to Seq. ID Nos. 1 or 2.
2. A polynucleotide according to claim 1 which is the mycobacterial gene SGS1 or a fragment thereof.
3. A polynucleotide according to claims 1 or 2 which comprises Seq.ID. Nos 1 or 2 or a fragment thereof.
4. A polynucleotide probe which comprises a fragment of at least 15 nucleotides of a polynucleotide as defined in claims 1, 2 or 3, optionally carrying a revealing label.
5. A vector carrying a polynucleotide as defined in claims 1, 2 or 3.
6. A polypeptide in substantially isolated form derived from a polynucleotide according to claim 1 or a vector according to claim 5, or a fragment of said polypeptide.
7. A polypeptide in substantially isolated form which comprises any one of the sequences set out in Seq ID.Nos 3 and 4 or a polypeptide substantially homologous thereto, or a fragment thereof.
8. An antibody capable of binding a polypeptide or fragment thereof as defined in claims 6 and 7.
9. A test kit for detecting the presence or absence of a pathogenic mycobacterium in a sample which comprises a polynucleotide according to any one of claims 1 to 4, a polypeptide according to claim 5 to 7, or an antibody according to claim 8.

10. A method of detecting the presence or absence of antibodies in an animal or human, against a pathogenic mycobacteria in a sample which comprises:
 - (a) providing a polypeptide according to claims 6 or 7 comprising an epitope;
 - (b) incubating a biological sample with said polypeptide under conditions which allow for the formation of an antibody-antigen complex; and
 - (c) determining whether antibody-antigen complex comprising said polypeptide is formed.
11. A method of detecting the presence or absence of a polypeptide according to claims 6 or 7 in a biological sample which method which comprises:
 - (a) providing an antibody according to claim 8;
 - (b) incubating a biological sample with said antibody under conditions which allow for the formation of an antibody-antigen complex; and
 - (c) determining whether antibody-antigen complex comprising said antibody is formed.
12. A method of detecting the presence or absence of cell mediated immune reactivity in an animal or human, to a polypeptide according to claims 6 or 7 which method comprises
 - (a) providing a polypeptide according to claims 6 or 7 comprising an epitope;.
 - (b) incubating a cell sample with said polypeptide under conditions which allow for a cellular immune response such as release of cytokines or other mediator or reaction to occur; and
 - (c) detecting the presence of said cytokine or mediator or cellular response in the incubate.
13. A pharmaceutical composition comprising a polypeptide according to claim 6 or 7 in a suitable carrier or diluent.
14. A composition according to claim 13 for use in the treatment or prevention of diseases caused by mycobacteria.

15. A method of treating or preventing mycobacterial disease in an animal or human caused by mycobacteria which express a polypeptide according to claim 6, which method comprises vaccinating or treating an animal or human with an effective amount of said polypeptide.
16. A method of treating or preventing mycobacterial diseases in animals or humans caused by mycobacteria containing the polynucleotide SGS1, which method comprises vaccinating or treating an animal or human with an effective amount of a polynucleotide according to claims 1 to 5.
17. A method according to claims 16 or 17 for increasing the in vivo susceptibility of mycobacteria to antimicrobial drugs.

Figure 1 Seq. ID No. 1
Identifier sequence for the polynucleotide encoded by the positive strand of the
mycobacterial gene SGS1

```
5' - 1 GATCCAAC TA AACCCGATGG AACCCCGCGC AAAC TATTGG ACGTCTCCGC GCTACGCAGT
      61 TGGGTTGGCG CCCGCGAATC GCACTGAAAG AGGGCATCGA TGCAACGGTG TCGTGGTACC
      121 GCACAAATGC CGATGCCGTG AGGAGGTAAA GCTGCGGGCC GGCCGATGTT ATCCCTCCGG
      181 CCGGACGGGT AGGGCGACCT GCCATCGAGT GGTACGGCAG TCGCCTGGCC GGCGAGGCGC
      241 ATGGCCTATG TGAGTATCCC ATAGCCTGGC TTGGCTCGCC CCTACGCATT ATCAGTTGAC
      301 CGCTTTCGCG CCACGTCGCA GGCTTGCGGC AGCATCCCGT TCAGGTCTCC TCATGGTCCG
      361 GTGTGGCACG ACCACGCAAG CTCGAACCGA CTCGTTTCCC AATTTCGCAT GCTAATATCG
      421 CTCGATGGAT TTTTTCGCA ACGCCGGCTT GATGGCTCGT AACGTTAGCA CCGAGATGCT
      481 GCGCCACTCC GAACGAAAGC GCCTATTAGT AAACCAAGTC GAAGCATACG GAGTCAACGT
      541 TGTTATTGAT GTCGGTGCTA ACTCCGGCCA GTTCGGTAGC GCTTTGCGTC GTGCAGGATT
      601 CAAGAGCCGT ATCGTTTCCT TTGAACCTCT TTCGGGGCCA TTTGCGCAAC TAACGCGCAA
      661 GTCGGCATCG GATC -3'
```

Figure 2. Seq. ID No. 2

Identifier sequence for the polynucleotide encoded by the complementary strand of the mycobacterial gene SGS1

```
5' - 1 GATCCGATGC CGACTTGCGC GTTAGTTGCG CAAATGGCCC CGAAAGAGGT TCAAAGGAAA
      61 CGATACGGCT CTTGAATCCT GCACGACGCA AAGCGCTACC GAACTGGCCG GAGTTAGCAC
     121 CGACATCAAT AACAACGTTG ACTCCGTATG CTTCGACTTG GTTTACTAAT AGGCGCTTTC
     181 GTTCGGAGTG GCGCAGCATC TCGGTGCTAA CGTTACGAGC CATCAAGCCG GCGTTGCGCA
     241 AAAAATCCAT CGAGCGATAT TAGCATGCGA AATTGGGAAA CGAGTCGGTT CGAGCTTGCG
     301 TGGTCGTGCC ACACCGGACC ATGAGGAGAC CTGAACGGGA TGCTGCCGCA AGCCTGCGAC
     361 GTGGCGCGAA AGCGGTCAAC TGATAATGCG TAGGGGCGAG CCAAGCCAGG CTATGGGATA
     421 CTCACATAGG CCATGCGCCT CGCCGGCCAG GCGACTGCCG TACCACTCGA TGGCAGGTCG
     481 CCCTACCCGT CCGGCCGGAG GGATAACATC GGCCGGCCCG CAGCTTTACC TCCTCACGGC
     541 ATCGGCATTT GTGCGGTACC ACGACACCGT TGCATCGATG CCCTCTTTCA GTGCGATTTC
     601 CGGGCGCCAA CCCAACTGCG TAGCGCGGAG ACGTCCAATA GTTTGCGCGG GGTTCATCG
     661 GGTTTAGTTG GATC -3'
```


Figure 3. Seq. ID No. 3
Identifier sequences for the polypeptides encoded by the positive strand of the mycobacterial gene SGS1

```

NH2-
Frame +3   SerAsnOchThrArgTrpAsnProAlaGlnThrIleGlyArgLeuArgAlaThrGlnLeu
Frame +2   IleGlnLeuAsnProMetGluProArgAlaAsnTyrTrpThrSerProArgTyrAlaVal
Frame +1   AspProThrLysProAspGlyThrProArgLysLeuLeuAspValSerAlaLeuArgSer
          1   GATCCAACATAACCCGATGGAACCCGCGCAACTATTGGACGTCTCCGCGCTACGCAGT

          +3   GlyTrpArgProArgIleAlaLeuLysGluGlyIleAspAlaThrValSerTrpTyrArg
          +2   GlyLeuAlaProAlaAsnArgThrGluArgGlyHisArgCysAsnGlyValValValPro
          +1   TrpValGlyAlaArgGluSerHisUmbLysArgAlaSerMetGlnArgCysArgGlyThr
          61   TGGGTTGGCGCCCGCAATCGCACTGAAAGAGGGCATCGATGCAACGGTGTCTGGTACC

          +3   ThrAsnAlaAspAlaValArgArgOchSerCysGlyProAlaAspValIleProProAla
          +2   HisLysCysArgCysArgGluGluValLysLeuArgAlaGlyArgCysTyrProSerGly
          +1   AlaGlnMetProMetProUmbGlyGlyLysAlaAlaGlyArgProMetLeuSerLeuArg
          121  GCACAAATGCCGATGCCGTGAGGAGGTAAAGCTGCGGGCCGGCCGATGTTATCCCTCCGG

          +3   GlyArgValGlyArgProAlaIleGluTrpTyrGlySerArgLeuAlaGlyGluAlaHis
          +2   ArgThrGlyArgAlaThrCysHisArgValValArgGlnSerProGlyArgArgGlyAla
          +1   ProAspGlyAmbGlyAspLeuProSerSerGlyThrAlaValAlaTrpProAlaArgArg
          181  CCGGACGGGTAGGGCGACCTGCCATCGAGTGGTACGGCAGTCGCCTGGCCGGCGAGGCGC

          +3   GlyLeuCysGluTyrProIleAlaTrpLeuGlySerProLeuArgIleIleSerUmbPro
          +2   TrpProMetUmbValSerHisSerLeuAlaTrpLeuAlaProThrHisTyrGlnLeuThr
          +1   MetAlaTyrValSerIleProAmbProGlyLeuAlaArgProTyrAlaLeuSerValAsp
          241  ATGGCCTATGTGAGTATCCCATAGCCTGGCTTGGCTCGCCCCTACGCATTATCAGTTGAC

          +3   LeuSerArgHisValAlaGlyLeuArgGlnHisProValGlnValSerSerTrpSerGly
          +2   AlaPheAlaProArgArgArgLeuAlaAlaAlaSerArgSerGlyLeuLeuMetValArg
          +1   ArgPheArgAlaThrSerGlnAlaCysGlySerIleProPheArgSerProHisGlyPro
          301  CGCTTTCGCGCCACGTCGCGAGGCTTGGCGCAGCATCCCGTTACGGTCTCTCATGGTCCG

          +3   ValAlaArgProArgLysLeuGluProThrArgPheProIleSerHisAlaAsnIleA
          +2   CysGlyThrThrThrGlnAlaArgThrAspSerPheProAsnPheAlaCysOchTyrAr
          +1   ValTrpHisAspHisAlaSerSerAsnArgLeuValSerGlnPheArgMetIleSer
          361  GTGTGGCACGACCACGAAGCTCGAACCGACTCGTTTCCCAATTTTCGATGCTAATATCG

          +3   laArgTrpIlePheCysAlaThrProAlaUmbTrpLeuValThrLeuAlaProArgCysC
          +2   gSerMetAspPheLeuArgAsnAlaGlyLeuMetAlaArgAsnValSerThrGluMetLe
          +1   LeuAspGlyPhePheAlaGlnArgArgLeuAspGlySerOchArgAmbHisArgAspAla
          421  CTCGATGGATTTTTTGGCGCAACGCCGGCTTGATGGCTCGTAACGTTAGCACCGAGATGCT

          +3   ysAlaThrProAsnGluSerAlaTyrAmbOchThrLysSerLysHisThrGluSerThrLeu
          +2   uArgHisSerGluArgLysArgLeuLeuValAsnGlnValGluAlaTyrGlyValAsnVal
          +1   AlaProLeuArgThrLysAlaProIleSerLysProSerArgSerIleArgSerGlnArg
          481  GCGCCACTCCGAACGAAAGCGCCTATTAGTAAACCAAGTCGAAGCATACGGAGTCAACGT

          +3   LeuLeuMetSerValLeuThrProAlaSerSerValAlaLeuCysValValGlnAspSer
          +2   ValIleAspValGlyAlaAsnSerGlyGlnPheGlySerAlaLeuArgArgAlaGlyPhe
          +1   CysTyrUmbCysArgCysOchLeuArgProValArgAmbArgPheAlaSerCysArgIle
          541  TGTTATTGATGTCGGTGCTAACTCCGGCCAGTTCGGTAGCGCTTTGCGTCTGTCAGGATT

+3   ArgAlaValSerPheProLeuAsnLeuPheArgGlyHisLeuArgAsnOchArgAlaSer
      +2   LysSerArgIleValSerPheGluProLeuSerGlyProPheAlaGlnLeuThrArgLys
      +1   GlnGluProTyrArgPheLeuUmbThrSerPheGlyAlaIleCysAlaThrAsnAlaGln
      601  CAAGAGCCGTATCGTTTCCTTTGAACCTCTTCGGGGCCATTTGCGCAACTAACGCGCAA

      +3   ArgHisArgIle
      +2   SerAlaSerAsp   -COOH
      +1   ValGlyIleGly
      661  GTCGGCATCGGATC   -3'

```

Figure 4. Seq. ID No. 4
 Identifier sequences for the polypeptides encoded by the complementary strand of
 the mycobacterial gene SGS1

```

NH2-
Frame +3   SerAspAlaAspLeuArgValSerCysAlaAsnGlyProGluArgGlySerLysGluThr
Frame +2   IleArgCysArgLeuAlaArgAmbLeuArgLysTrpProArgLysArgPheLysGlyAsn
Frame +1   AspProMetProThrCysAlaLeuValAlaGlnMetAlaProLysGluValGlnArgLys
5'- 1      GATCCGATGCCGACTTGCGCGTTAGTTGCGCAATGGCCCCGAAAGAGGTTCAAAGGAAA

+3   IleArgLeuLeuAsnProAlaArgArgLysAlaLeuProAsnTrpProGluLeuAlaPro
+2   AspThrAlaLeuGluSerCysThrThrGlnSerAlaThrGluLeuAlaGlyValSerThr
+1   ArgTyrGlySerUmbIleLeuHisAspAlaLysArgTyrArgThrGlyArgSerAmbHis
61   CGATACGGCTCTTGAATCCTGCACGACGCAAAGCGCTACCGAACTGGCCGGAGTTAGCAC

+3   ThrSerIleThrThrLeuThrProTyrAlaSerThrTrpPheThrAsnArgArgPheArg
+2   AspIleAsnAsnAsnValAspSerValCysPheAspLeuValTyrOchAmbAlaLeuSer
+1   ArgHisGlnOchGlnArgUmbLeuArgMetLeuArgLeuGlyLeuLeuIleGlyAlaPhe
121  CGACATCAATAACAACGTTGACTCCGTATGCTTCGACTTGGTTTACTAATAGGCGCTTTC

+3   SerGluTrpArgSerIleSerValLeuThrLeuArgAlaIleLysProAlaLeuArgLys
+2   PheGlyValAlaGlnHisLeuGlyAlaAsnValThrSerHisGlnAlaGlyValAlaGln
+1   ValArgSerGlyAlaAlaSerArgCysOchArgTyrGluProSerSerArgArgCysAla
181  GTTCGGAGTGGCGCAGCATCTCGGTGCTAACGTTACGAGCCATCAAGCCGGCGTTGCGCA

+3   LysSerIleGluArgTyrAmbHisAlaLysLeuGlyAsnGluSerValArgAlaCysVal
+2   LysIleHisArgAlaIleLeuAlaCysGluIleGlyLysArgValGlySerSerLeuArg
+1   LysAsnProSerSerAspIleSerMetArgAsnTrpGluThrSerArgPheGluLeuAla
241  AAAAATCCATCGAGCGATATTAGCATGCGAAATTGGGAAACGAGTCGGTTTCGAGCTTGCG

+3   ValValProHisArgThrMetArgArgProGluArgAspAlaAlaAlaSerLeuArgArg
+2   GlyArgAlaThrProAspHisGluGluThrUmbThrGlyCysCysArgLysProAlaThr
+1   TrpSerCysHisThrGlyProUmbGlyAspLeuAsnGlyMetLeuProGlnAlaCysAsp
301  TGGTCGTGCCACACCGGACCATGAGGAGACCTGAACGGGATGCTGCCGAAGCCTGCGAC

+3   GlyAlaLysAlaValAsnUmbOchCysValGlyAlaSerGlnAlaArgLeuTrpAspThr
+2   TrpArgGluSerGlyGlnLeuIleMetArgArgGlyGluProSerGlnAlaMetGlyTyr
+1   ValAlaArgLysArgSerThrAspAsnAlaAmbGlyArgAlaLysProGlyTyrGlyIle
361  GTGGCGCGAAAGCGGTCAACTGATAATGCGTAGGGGCGAGCCAAGCCAGGCTATGGGATA

+3   HisIleGlyHisAlaProArgArgProGlyAspCysArgThrThrArgTrpGlnValAla
+2   SerHisArgProCysAlaSerProAlaArgArgLeuProTyrHisSerMetAlaGlyArg
+1   LeuThrAmbAlaMetArgLeuAlaGlyGlnAlaThrAlaValProLeuAspGlyArgSer
421  CTCACATAGGCCATGCGCCTCGCCGGCCAGGCGACTGCCGTACCACTCGATGGCAGGTGC

+3   LeuProValArgProGluGlyOchHisArgProAlaArgSerPheThrSerSerArgHis
+2   ProThrArgProAlaGlyGlyIleThrSerAlaGlyProGlnLeuTyrLeuLeuThrAla
+1   ProTyrProSerGlyArgArgAspAsnIleGlyArgProAlaAlaLeuProProHisGly
481  CCCTACCCGTCCGGCCGGAGGATAACATCGGCCGGCCCGCAGCTTACCTCCTCACGGC

+3   ArgHisLeuCysGlyThrThrThrProLeuHisArgCysProLeuSerValArgPheAla
+2   SerAlaPheValArgTyrHisAspThrValAlaSerMetProSerPheSerAlaIleArg
+1   IleGlyIleCysAlaValProArgHisArgCysIleAspAlaLeuPheGlnCysAspSer
541  ATCGGCATTTGTGCGGTACCACGACACCGTTGCATCGATGCCCTCTTTCAGTGCGAT

+3   GlyAlaAsnProThrAlaAmbArgGlyAspValGlnAmbPheAlaArgGlySerIleGly
+2   GlyArgGlnProAsnCysValAlaArgArgArgProIleValCysAlaGlyPheHisArg
+1   ArgAlaProThrGlnLeuArgSerAlaGluThrSerAsnSerLeuArgGlyValProSer
601  CGGGCGCCAACCAACTGCGTAGCGCGGAGACGTCCAATAGTTTGC GCGGGGTTCCATCG

+3   PheSerTrpIle
+2   ValAmbLeuAsp   -COOH
+1   GlyLeuValGly
661  GGTTTAGTTGGATC -3'

```

Specificity PCR Results

| SPECIES | SGS1 | IS900 | IS902 |
|---|------|-------|-------|
| <i>M. paratuberculosis</i> (Map) | | | |
| 0025 (bovine, CVL Weybridge) | + | + | - |
| 0021 (caprine, Moredun) | + | + | - |
| 0022 (bovine, Moredun) | + | + | - |
| 0139 (human, Chiodini <i>et al.</i> 1984*) | + | + | - |
| 0209 (bovine, Whipple <i>et al.</i> 1990 ^b) | + | + | - |
| 0208 | + | + | - |
| 0211 | + | + | - |
| 0210 | + | + | - |
| 0212 | + | + | - |
| 0207 | + | + | - |
| 0204 | + | + | - |
| 0206 | + | + | - |
| <i>M. avium</i> sub.sp. <i>para. progen.</i> (Mavpp) | | | |
| 0033 (AIDS, Hoffner) | + | + | - |
| <i>M. avium</i> sub.sp. <i>silvaticum</i> (Mas) | | | |
| 0010 (wood pigeon, Thorel) | + | - | + |
| 0012 (wood pigeon, Thorel) | + | - | + |
| <i>M. avium</i> (Maa) | | | |
| 0018 (armadillo, Portaels) | - | - | - |
| 0040 (AIDS, Hoffner) | - | - | - |
| 0038 | - | - | - |
| 0037 | - | - | - |
| 0034 | - | - | - |
| <i>M. malmoense</i> | - | ND | ND |
| <i>M. szulgai</i> | - | ND | ND |
| <i>M. goodii</i> | - | ND | ND |
| <i>M. chelonae</i> | - | ND | ND |
| <i>M. fortuitum</i> | - | ND | ND |
| <i>M. bovis</i> | - | ND | ND |
| <i>M. tuberculosis</i> | - | ND | ND |
| <i>M. leprae</i> | - | ND | ND |
| <i>M. phlei</i> | - | ND | ND |
| <i>E. coli</i> | - | ND | ND |
| <i>S. aureus</i> | - | ND | ND |
| <i>Nocardia</i> sp. | - | ND | ND |
| <i>Streptococcus</i> sp. | - | ND | ND |
| <i>Shigella</i> sp. | - | ND | ND |
| <i>Pseudomonas</i> sp. | - | ND | ND |

* ND = No t Done

* Chiodini, R., van Kruiningen, H.J., Merkal, R.S., Thayer, W.R. and Couteau, J.A. (1984). Characteristics of an unclassified *Mycobacterium* species isolated from patients with Crohn's disease. *Journal of Clinical Microbiology* 20, 966-971.^b Whipple, D., Kapke, P. and Vary, C. (1990). Identification of restriction fragment length polymorphisms in DNA from *Mycobacterium paratuberculosis*. *Journal of Clinical Microbiology* 28, 2561-2564.

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